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54 Enzyme inhibitors.

57 Preparation of renin inhibitors based on the structure of natural renin substrate at residues 6 to 13 from the amino terminal thereof, the inhibitors being polypeptide analogues having in particular an isosteric non-peptide link corresponding to the 10, 11 peptide link of the substrate, and preparation of dipeptide analogues.

EP 0 045 665 A1

ENZYME INHIBITORS

The invention relates to renin-inhibiting peptide analogues.

BACKGROUND

Renin is a natural enzyme, disorders in relation to which are implicated in many cases of hypertension. It is released into the blood from the kidney, and cleaves from a blood glycoprotein a decapeptide known as angiotensin-I. Circulating angiotensin-I is cleaved in lung, kidney and other tissues to an octapeptide, angiotensin-II, which raises blood pressure both directly by causing arteriolar constriction and indirectly by stimulating release of the sodium-retaining hormone aldosterone from the adrenal gland and thus causing a rise in extracellular fluid volume. The latter effect is caused by angiotensin-II itself or a heptapeptide cleavage product angiotensin-III.

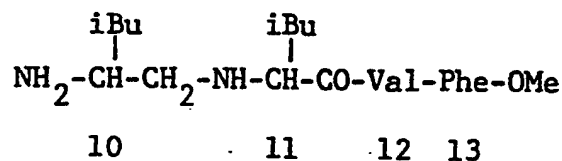
Inhibitors of renin have therefore been sought, with two ends in view, first the provision of a diagnostic agent for identification of cases of hypertension due to renin excess, and secondly the provision of an agent for control of hypertension in such cases.

The present inventors' approach has been to consider the peptide sequence characterising the natural renin substrate at its binding site, and to seek peptide analogues sufficiently similar to bind to the enzyme, in competition with the natural substrate, but sufficiently dissimilar to it to be cleaved slowly or not at all. Such analogues will block the action of the enzyme and attack the hypertension at source.

proposed by Kokubu, Nature, 217 456 (1968) but it is inactive in vivo, because of binding to plasma proteins and rapid attack by natural peptidases.

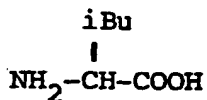
One of the present inventors undertook some years ago a development of Kokubu's work, seeking a renin inhibitor active in vivo, in which analogues of peptides similar to Kokubu's were made but having a methylene imino group  $-\text{CH}_2-\text{NH}-$  in place of the peptide link  $-\text{CO}-\text{NH}-$  between the leucine residues. One of these analogues was:

(III)



which is the tetrapeptide (I) modified at the Leu-Leu link, leucine of course being

(IV)



This analogue (III) was the first effective in-vivo inhibitor of renin and was shown to have significant antihypertensive action in Goldblatt hypertensive rats (Parry, Russell and Szelke p. 541 in "Chemistry and Biology of Peptides" Ed. Meienhofer, Ann Arbor Science Publishers 1972). Little or no attention has however been paid to the work, which the authors themselves were unable to pursue, in spite of considerable activity in the general field of substrate-based inhibitors for renin, reviewed for example by Haber & Burton, Federation Proc. 38 No. 13 2768-2773 (1979).

The present invention is a development of the above work. Behind it is a concept of modifying peptide structures related to the peptide sequence at the site of action of renin on the natural substrate, by isosteric substitution at, at least, the site of cleavage. Optionally further there is isosteric substitution or other modification at other positions to increase stability or to modify the properties of the final peptide, for example its solubility under physiological conditions or its resistance to in vivo exopeptidase attack. Such modification may for example be by incorporation of residues other than those of the natural L-amino acids; by protection of the N-terminus with acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl or other groups; or by conversion of the C-terminal carboxyl to another functional group, e.g. the corresponding alcohol, present as such or in ether or ester form.

General reference to amino acids and amino acyl residues and side chains in both the description and claims herein is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L- forms, and amino is to be taken as including imino except where an aromatic acid, residue or side chain is specified.

The compounds of the present invention, showing desirable renin inhibitory action, are of the general formula:



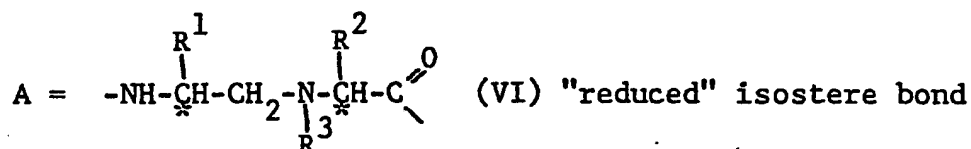
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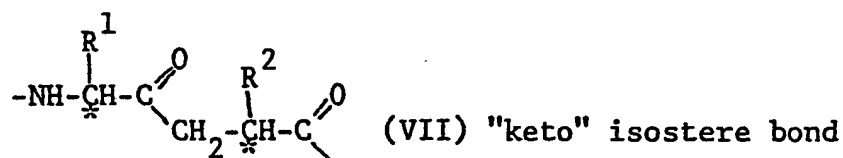
where Pro, Phe and His may be in substituted form;

X = H; or an acyl or other N-protecting group e.g.  
acetyl, pivaloyl, t-butyloxycarbonyl (Boc), benzoyl  
or lower alkyl (primarily C<sub>1</sub>-C<sub>5</sub>); or an L- or D-  
amino- acyl residue, which may itself be  
N-protected similarly;

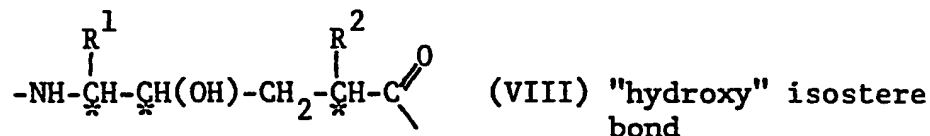
Y = D- or L-His or other D- or L- basic or aromatic  
amino-acyl residue, or is absent;



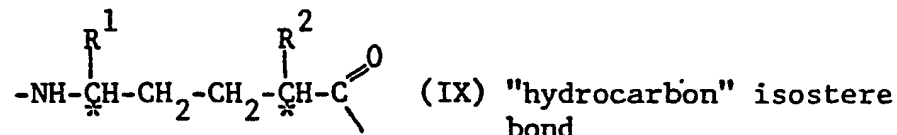
or



or



or



where the configuration at asymmetric centres \* is either

R or S, where in VIII the hydroxy group may be present  
as such or protected in ether -OR<sup>4</sup> or ester -O-C(=O)-R<sup>4</sup> form

where R<sup>4</sup> is as given under W below and where

R<sup>1</sup> and R<sup>2</sup>, the same or different = <sup>i</sup>Pro (isopropyl),

<sup>i</sup>Bu (isobutyl), Bzl (benzyl) or other lipophilic  
or aromatic amino-acid side chain

$R^3 = -H$ ; lower alkyl ( $C_1-C_5$ ); or  $-SO_2Ph$ ,  $-SO_2C_6H_4CH_3(p)$ ,  
Boc, formyl or other N-protecting group;

B = D- or L- Val or Ile or other D- or L- lipophilic amino-  
acyl residue;

Z = D- or L- Tyr, Phe, His or other L- or D- aromatic amino-  
acyl residue;

and

W =  $-OH$  as such or in protected ester form as  $-OR^4$  where

$R^4 =$  lower alkyl primarily  $C_1-C_5$  and particularly

<sup>t</sup>Bu, or cycloalkyl primarily  $C_3-C_7$ , or Bzl, or other  
ester forming group; or  $-NH_2$  as such or in protected  
amide form as  $-NHR^5$  or  $-N(R^5)_2$  (where  $R^5 =$  an

N-protecting or other substituent group e.g. lower  
alkyl as for  $R^4$  and  $(R^5)_2 =$  two such or e.g. cyclo-  
alkyl, primarily  $C_3-C_7$ ) or as  $-NH-(CH_2)_n-Q$  or

$-NR^5-(CH_2)_n-Q$  (where  $n = 2$  to  $6$  and  $Q = NH_2$  or

$-NH-C \begin{array}{l} \nearrow NH \\ \searrow NH_2 \end{array}$  and wherein any of the hydrogens attached

to nitrogen may be substituted by  $R^5$  or  $(R^5)_2$ ;

an L- or D- serine or lysine, arginine or other basic  
amino-acyl residue as such or in amide form substituted  
amide form or ester form e.g. containing a group or  
groups as given for  $R^4$  and  $R^5$  above as the case

may be; or an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for  $R^4$  above

or

L- or D-

Z + W = an alcohol derived from/Tyr, Phe, His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced,  $-\text{CH}_2-\text{NH}-$ , keto,  $-\text{C}(=\text{O})-\text{CH}_2-$ , hydroxy,  $-\text{CH}(\text{OH})-\text{CH}_2-$ , or hydrocarbon,  $-\text{CH}_2-\text{CH}_2-$  isosteric links and further being in free form or in protected or salt form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups, in particular as their physiologically acceptable acid addition salts at basic centres.

The above compounds may in particular be those related to the substrate sequence in the horse (B = Val at position 12) or those related to the substrate sequence in man (B = Ile at position 12). Particular groups of these compounds are set out in claims 2 and 3 respectively herein, as formulae VA and VB to which reference may be made but which are not repeated at this point.

The numbering of residues in formulae (V), (VA) and (VB) shows the correspondence with the renin substrates themselves, but without limitation of the generality of the formulae.

Where a peptide bond in addition to that corresponding to the Leu-Leu or Leu-Val bond in the natural renin substrate is isosterically substituted, the 7,8 and 8,9 positions i.e. the Pro-Phe and Phe-His bonds in formula V are preferred,



or possibly both of these positions, and it is further preferred that the substitution should be



where  $\text{R}_3$  is as set out above. The alternative isosteric substitutions set out herein may however be used.

Protective or substituent groupings as mentioned above may be any of these known in the polypeptide art, amply disclosed in the literature and not requiring discussion at length here. Generally the selection of the 'protective' groups is according to their function, some being primarily intended to protect against undesired reaction during synthetic procedures while the N- and C- terminal substituents are for example directed against the attack of exopeptidases on the final compounds or to increase their solubility and hence physiological acceptability.

It is in particular possible for one or more remaining peptide bonds in the compounds of formula (V), (VA) or (VB) to be N-substituted with protective groups.

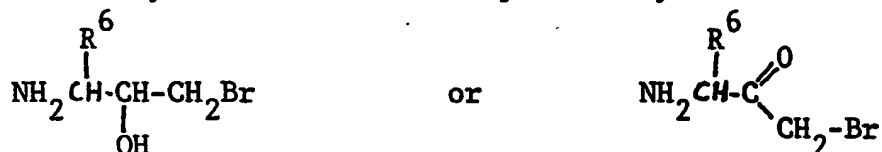
The invention further lies i) In a diagnostic test for high renin states, blood pressure falling most when renin is high, and as a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), the administration of a polypeptide analogue as above followed by monitoring of blood pressure, and such polypeptide analogues when for such use, and

ii) In the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, the administration of a renin-inhibiting amount of a polypeptide analogue as above, and such polypeptide analogues when for such use.

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The long and short term response of blood pressure to renin inhibitors is predictive of surgical outcome. In all cases single and repeated doses and any conventional form of pharmaceutical composition may be used, for administration by intranasal or oral route, injection, or any other means as convenient. Amounts may for example be 0.001 to 10 mg/kg body weight daily more usually 0.01 to 1mg, according to the potency of the analogue and the severity of the condition. Dosage unit compositions may contain such amounts or submultiples thereof to make up the daily dose. (Dosages herein and in the claims are related to the free base content where compounds are in salt form.)

The invention still further extends to a product and method of making a hydroxy or keto isostere of a dipeptide wherein a derivative of a halohydrin preferably a bromohydrin or haloketone preferably a bromoketone



wherein  $\text{R}^6$  is an amino acid side chain and the  $\text{NH}_2$  and OH groups are in protected form is subjected to an alkylation procedure to attach a group  $\text{R}^7$

$-\text{CH}-\text{COOH}$  and gives the desired isostere as such or in protected form,  $\text{R}^7$  being the same or a different amino acid side chain.

In particular the alkylation procedure may be

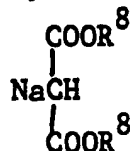
- i) by reaction with an alkali metal carboxylic acid derivative preferably a lithium derivative



where  $\text{R}^7$  is as above.

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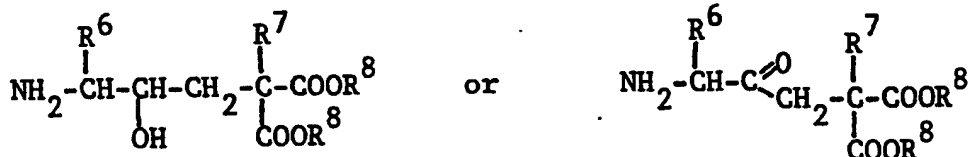
- ii) by reaction with an alkali metal malonic ester derivative preferably a sodium derivative



where  $R^8$  is an esterifying group and a halide preferably an iodide



where  $R^7$  is as above to give intermediate



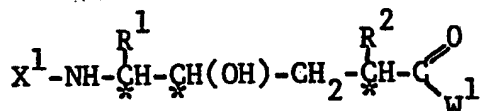
in protected form which intermediate is then decarboxylated and if desired deprotected to give the desired isostere



as such or in protected form.

The hydroxy isosteres so produced may further be oxidised to the corresponding keto isosteres, and such method and the isosteres produced fall within the invention.

In particular the methods may be applied to the production of a hydroxy dipeptide isostere of the formula



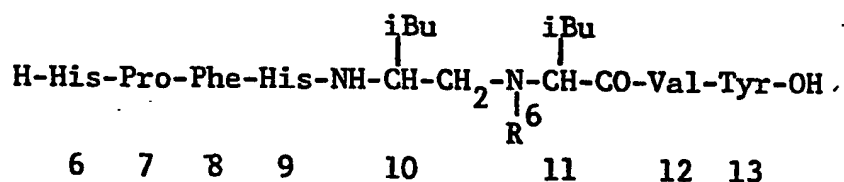
or the corresponding keto isosteres, where the significance of \*,  $X^1$  and  $W^1$  is as above except that  $X^1$  and  $W^1$  do not represent amino-acyl.

The dipeptide isosteres given by all these methods may be incorporated in higher peptide analogues by the methods herein described or by the methods of peptide synthesis as generally known in the art, and the invention extends to the dipeptide whether as such or in the form of said higher analogues, in all cases as the compound itself or in protected form.

analogue

The dipeptide/syntheses are illustrated in detail herein, in the course of illustrating the preparation of the octapeptides and related compounds to which the invention chiefly relates.

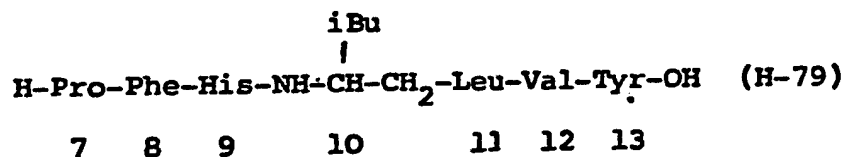
Specific analogues within the invention, all as such or in protected form, are



(H-76 where  $\text{R}^6$  = hydrogen; H-78 where  $\text{R}^6$  =  $\text{SO}_2\text{Ph}$ )

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and the corresponding analogue (H-77) with  $R^6$  = hydrogen and D-His at position 6. A further analogue, with the same methylene-imino isosteric replacement of a Leu-Leu peptide bond, is:



Further analogues within formula (VA) are given in the present disclosure in Examples VI to IX, XI and XII. Analogues within formula (VB) are given in Examples V and X.

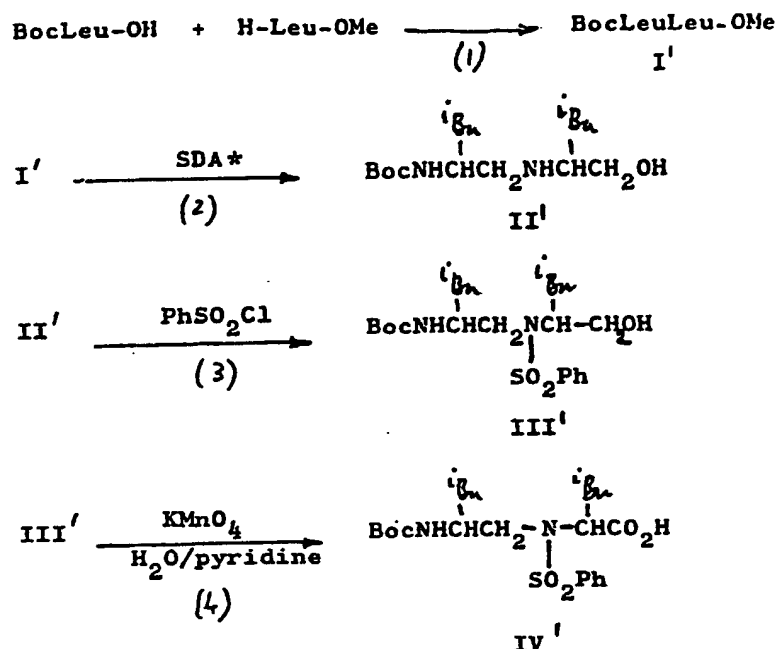
#### SYNTHETIC METHODS

The inventors have developed synthetic methods for the isosteric replacement of the peptide bond  $\text{-CO-NH-}$  with alternative groups, specifically  $\text{-CH}_2\text{-NH-}$  (reduced),  $\text{-CH}_2\text{CH}_2\text{-}$  (hydrocarbon),  $\text{-C}\begin{smallmatrix} \text{O} \\ \text{<} \end{smallmatrix}\text{CH}_2\text{-}$  (keto) and  $\text{-CH(OH)-CH}_2\text{-}$  (hydroxy) isosteres (see, e.g. Szelke, et al, pp. 57-70 in "Molecular Endocrinology" Vol. 1, Editors: MacIntyre and Szelke, Elsevier, Amsterdam 1977, and Hudson, Sharpe and Szelke, U.S. Patent 4 198 398 "Enkephalin Analogues").

Reference may be made to these publications for general discussion of such isosteric replacement. A reaction sequence for the preparation in particular of the reduced isostere of leucyl leucine for incorporation in the analogues disclosed herein is however for example:

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## Scheme 1

Synthesis of the protected reduced isostere of L-leucyl-L-leucine

\* Sodium di(methoxyethoxy) aluminium hydride

(1) Boc-Leucyl-leucine methyl ester

The dipeptide I' was prepared from Boc-leucine.H<sub>2</sub>O (27.5g, 0.11 mole) and leucine methyl ester .HCl (20g, 0.11 mole) by mixed anhydride coupling using N-methyl morpholine and isobutylchloroformate. After a standard work-up procedure the dipeptide I' was obtained as white needles, 35.0g (88%) from EtOAc/petrol bpt 40-60°, m.p. 132-133°.

(2) Preparation of compound II'

The dipeptide I' (7.2g, 20 mmole) was dissolved in benzene (120 ml, Na-dried). A solution of sodium dihydro-bis-(2-methoxyethoxy)aluminate (SDA, 70% in toluene, 41 ml) was added slowly with cooling. After addition, the solution was refluxed for ½ hr, cooled and poured into 0.5M ice-cold citric acid solution. At pH 2.5 the aqueous solution was extracted with ether (4X) and the combined extracts were discarded. The

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pH was adjusted to 9 with  $\text{Na}_2\text{CO}_3$  solution and the aqueous solution was saturated with sodium chloride. Extraction with ether (4X), followed by drying ( $\text{Na}_2\text{SO}_4$ ) of the combined organic phases, evaporation and crystallisation from petrol (40-60°) at -20° gave the reduced dipeptide  $\text{II}^1$ : 5.1g (78%) as white needles.

m.p. 59-60°

Nmr ( $\text{CDCl}_3$ ) 9.05 - 9.15 (12H, d, 4 x  $\text{CH}_3$ )  
 8.75 (6H, m, 2 x  $(\text{CH}_3)_2\text{CH}-\text{CH}_2$ ): 8.55 (9H, s,  $(\text{CH}_3)_3\text{CO}$ );  
 7.35 (5H, m,  $\text{CH}_2\text{NH}$ ,  $\text{CH}_2\text{OH}$ ); 6.05 - 6.85 (3H, m, 2 x  $\alpha\text{-CH}$  and  $\text{CH}_2\text{OH}$ ); 5.3 (1H, d, Boc  $\text{NH-}$ ).

(3) Protection of compound  $\text{II}^1$  with benzenesulphonyl.

The reduced compound  $\text{II}^1$  (11.0g, 34.7 mmole) in dioxan (100ml) was added to a solution of  $\text{KHCO}_3$  (21g., 6 equiv.) in  $\text{H}_2\text{O}$  (100ml). This mixture was cooled in ice and benzene-sulphonyl chloride (9.0ml, 2 equiv.) added in dioxan (25 ml) with vigorous stirring. Stirred at 22° overnight. Poured into ether, washed with 2N  $\text{NH}_4\text{OH}$  (4X),  $\text{H}_2\text{O}$  (1X) 0.5M. citric acid (2X to remove any unsulphonated material),  $\text{H}_2\text{O}$  (1X).

The protected compound  $\text{III}^1$  was obtained as an oil. Nmr spectroscopy showed the presence of one benzenesulphonyl group. This material was used without further purification in the next stage:-

(4) Oxidation of compound  $\text{III}^1$

The material from the preceding preparation was taken up in pyridine (50ml), cooled in ice and  $\text{KMnO}_4$  (11.0g 70 mmole) in  $\text{H}_2\text{O}$  (50 ml) and pyridine (100 ml) added. Stirred for 42 hrs at 20°. The  $\text{MnO}_2$  precipitate was removed and the filtrate diluted with citric acid solution until acidic. Ether extraction at pH 5 removed product and starting material. The product  $\text{IV}^1$  was obtained by (i)  $\text{NaHCO}_3$  extraction - to remove strongly acidic

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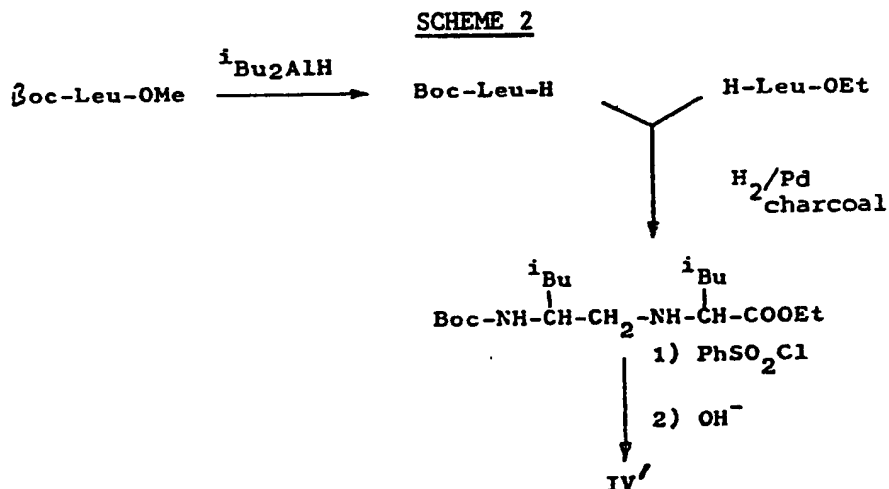
by-products (ii) extracted with 30% v/v 0.880 ammonia solution (6X). The ammonia washes contained essentially pure IV'. Starting material remaining in the ether was re-oxidised for 42 hrs and worked-up as above.

The total amount of IV' (isolated by acidifying the ammonia washes and extracting with  $\text{CHCl}_3$ ) obtained was 2.34g (20% based on II').

The material was a colourless foam Rf 0.41 by TLC on silica in benzene-dioxan-acetic acid (95:25:4).

Nmr ( $\text{CDCl}_3$ ): 8.9 - 9.3 (12H, m, 4 x  $\text{CH}_3$ ); 8.2 - 8.8 (15H, m,  $(\text{CH}_3)_3\text{CO}$  and 2 x  $(\text{CH}_3)_2\text{CH-CH}_2$ ); 5.4 - 7.0 (4H, m, 2 x  $\alpha\text{-CH}$  and  $\text{-CH}_2\text{-N-}$ ); \* 2.0 and 2.4 (5H, m,  $\text{C}_6\text{H}_5\text{SO}_2$ ), 1.2 (1H, br. s,  $\text{CO}_2\text{H}$ ).

Alternatively, the reduced Leu-Leu analogue IV may be synthesised by the following method:



The following is a synthetic method for a reduced Leu-Val isostere by a preferred route.





extracts were combined and dried ( $\text{Na}_2\text{SO}_4$ ). The resulting oil was passed rapidly through a pad of silica gel in 15% EtOAc/petrol 40-60°. The crude aldehyde was obtained as an oil, weight 18.68g. Nmr showed aldehyde content to be 85%, therefore yield of aldehyde: 15.9g (83%).

Nmr ( $\text{CDCl}_3$ ),  $\tau$  :

0.45 (1H, s,  $\text{CHO}$ ); 4.87 (H, br. d., Boc NH):

5.83 (1H, br. m., NH- $\text{CHCHO}$ ); 8.43-8.93 (12H, m,  $(\text{CH}_3)_3\text{C}$ ,  $(\text{CH}_3)_2\text{CHCH}_2$ ); 9.0 and 9.1 (12H, 2 x d,  $(\text{CH}_3)_2\text{CH}$ )

TLC: (solvent 30% EtOAc/petrol 60-80°),  $R_f = 0.43$ .

(2) Boc-L-Leucyl-L-valine benzyl ester reduced isostere, 2

L-Valine-OBzl (10 mmoles, from EtOAc/1N  $\text{NaHCO}_3$  partition of 3.8g of p-toluene sulphonate salt) and Boc-L-Leucinal (2.54g, 10 mmole aldehyde content) in dry tetrahydrofuran (20ml) stood over 5Å molecular sieve (10g) overnight. Sodium cyanoborohydride (630mg, 10mmoles) in MeOH (3ml) was added with cooling, then left at room temperature for 30 mins. The mixture was diluted with methylene chloride (100 ml), filtered and evaporated to dryness. The residue was passed down a silica column in 20% EtOAc/petrol (60-80°) to remove polar impurities. Isostere containing fractions were combined. Crystallisation from petrol 60-80° at -20° gave large clusters of needles, 1.52g (38%) m.p.

$\tau$  : 2.65 (5H, s,  $\text{OCH}_2\text{C}_6\text{H}_5$ );

6.35 (1H, m,  $\text{NHCHCO}_2\text{Bzl}$ ); 7.05 (1H, m, NH- $\text{CHCH}_2$ );

7.45 (2H, m,  $-\text{CH}_2\text{NH}-$ ); 8.25 - 8.90 (13H, m,  $(\text{CH}_3)_3\text{CO}-$ )

$(\text{CH}_3)_2\text{CHCH}_2$  and  $(\text{CH}_3)_2\text{CH}-$ ; 9.05 and 9.15

(12H, 2 x s, 2 x  $(\text{CH}_3)_2\text{CH}$ ).

TLC: (Solvent: 30% EtOAc/petrol 60/80°)  $R_f = 0.39$

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(3) N- (2S)-t-Butyloxycarbonylamino-4-methylpentyl, N-  
(3,4-dichlorobenzoyloxycarbonyl)-L-valine, 4

Boc-L-Leucyl-L-valine, benzyl ester-reduced-isostere (1.5g 3.68 mmoles) in dimethylformamide (60ml) was hydrogenated at STP over 5% Pd/C (150mg). After  $3\frac{1}{2}$  hrs. the colloidal solution was flushed with nitrogen and 1M NaOH (3.8ml, 1.05 equiv.) was added followed by 3,4-dichlorobenzyl pentachlorophenyl carbonate (1.92g, 4.07 mmoles). The mixture was kept at  $50^{\circ}$  in a stoppered flask for 24 hrs. and then evaporated to dryness. EtOAc was added and the Pd/C filtered off. The EtOAc solution was washed with 1M citric acid (2 x),  $H_2O$  (1 x), brine (1 x), and dried ( $Na_2SO_4$ ).

The crude isostere 4 was chromatographed on silica-gel (Merck Keisegel 60, 40-63  $\mu$ m) eluting with 2% MeOH/ $CHCl_3$  to give the title compound as a colourless oil.

Nmr( $CDCl_3$ ),  $\tau$  :

2.5 - 2.9 (3H, m,  $C_6Cl_2H_3$ ); 3.3 - 3.8 (2H, br, BocNH and  $CO_2H$ );  
 4.85 and 4.95 (2H, 2 x s,  $OCH_2-C_6Cl_2H_3$ ); 5.5 - 6.3 (2H, m, NHCHCH  
 and  $-NCHCO_2H$ ); 6.5 - 7.2 (2H br, 2 x d,  $CHCH_2N-$ ); 8.2 - 8.9  
 (13H, m,  $(CH_3)_3CO$ ,  $(CH_3)_2CHCH_2$  and  $(CH_3)_2CH-$ ); 8.9 - 9.4  
 (12H, m, 2 x  $(CH_3)_2CH$ )

TLC: (solvent 5% MeOH/ $CHCl_3$ )  $R_f$  = 0.32.

EXAMPLES

The following detailed Examples illustrate the invention.

The Examples are preceded by the preparation of Boc-Tyr [Bzl (2, 6 Cl<sub>2</sub>)] -O-resin. (Reaction times marked \* are convenient rather than necessary.)

Preparation of Resin

Boc-Tyr [Bzl(2,6 Cl<sub>2</sub>)] -OH (1.65g, 3.75mmol) was dissolved in ethanol (20ml) and water (5ml) added. The pH was brought to 7.0 with cesium bicarbonate solution and the solvent evaporated in vacuo. The residue was treated twice with toluene and evaporated to remove the last traces of water leaving a white powder which was dried for several hours over phosphorus pentoxide. The residue was dissolved in DMF (65 ml), chloromethylated resin (10g, 7.5 mequiv) added and the reaction stirred at 37° for four days.

The resin was then filtered and washed thoroughly with DMF, DMF/water (9:1) and then DMF again. The resin was then resuspended in DMF (65ml) and treated with acetic anhydride (2.36ml, 25mmol) and triethylamine (3.5ml, 25mmol) for 3 days.

The resin was filtered, washed thoroughly with DMF, DMF/water (9:1) and methanol and dried. The resin was then "defined" by shaking a suspension in dichloromethane and removing the particles slowest to float. The resin was then dried.

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Yield 10.8 g.

Amino-acid analysis: - (12N-HCl/propionic acid 1:1 130°, 2 hours)  
gave an incorporation of 0.11mmol/g.

Example I

H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH (H-76)

Boc-Tyr [Bzl(2,6 Cl<sub>2</sub>)] -O-Resin (3g, 0.6mmol)

was washed with reagents in the following sequence: CH<sub>2</sub>Cl<sub>2</sub> (3X)

iPrOH (2X), CH<sub>2</sub>Cl<sub>2</sub> (3X), 40% TFA/CH<sub>2</sub>Cl<sub>2</sub> 1 min then 20 min,  
CH<sub>2</sub>Cl<sub>2</sub> (3X), iPrOH (2X), CH<sub>2</sub>Cl<sub>2</sub> (3X), 40% TFA/CH<sub>2</sub>Cl<sub>2</sub> 1min  
then 20 min, CH<sub>2</sub>Cl<sub>2</sub> (3X) iPrOH (2X) CH<sub>2</sub>Cl<sub>2</sub> (3X), 10% Et<sub>3</sub>N/  
CH<sub>2</sub>Cl<sub>2</sub> (2 x 2min), CH<sub>2</sub>Cl<sub>2</sub> (5 X). Boc-Val-OH (0.65g, 3mmol)  
was then coupled using DCCI (0.68g, 3.3mmol) and HOBT (0.92g,  
6mmol) in DMF/CH<sub>2</sub>Cl<sub>2</sub> (1:1) for 17 hours. The resin was then  
washed with DMF (3X), CH<sub>2</sub>Cl<sub>2</sub> (3X), iPrOH (2X), CH<sub>2</sub>Cl<sub>2</sub> (3X)  
10% Et<sub>3</sub>N/CH<sub>2</sub>Cl<sub>2</sub> (2 min), CH<sub>2</sub>Cl<sub>2</sub> (5X) then acetylated using  
acetylimidazole (0.66g, 6mmol) in DMF for 1 hour. The resin  
was then washed with DMF (3X) CH<sub>2</sub>Cl<sub>2</sub> (3X) iPrOH (2X) and finally  
CH<sub>2</sub>Cl<sub>2</sub> (3X).

This sequence of washes and reactions was repeated  
for the addition of each of the residues with the following  
modifications.

After deprotection of the Boc-Val-Tyr [Bzl (2,6 Cl<sub>2</sub>)] -O-  
resin Boc-NH-CH (CH<sub>2</sub>CHMe<sub>2</sub>)-CH<sub>2</sub>-N(SO<sub>2</sub>Ph)-CH(CH<sub>2</sub>CHMe<sub>2</sub>)-CO<sub>2</sub>H  
(0.42g, 0.9mmol) was coupled using DCCI (0.28g, 1.35mmol) and  
HOBT (0.275g, 1.8 mmol) in DMF/CH<sub>2</sub>Cl<sub>2</sub> (1:1) for 18 hours,  
followed by acetylation using acetylimidazole (0.66g, 6mmol)  
in DMF for 1 hour.

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After deprotection, Boc-His(Dnp)-OH (1.44g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92g, 6mmol) for 2 hours, followed by acetylation for 1 hour.

Deprotection of the histidyl peptide was achieved using 50 % TFA/CH<sub>2</sub>Cl<sub>2</sub> instead of the usual 40% TFA/CH<sub>2</sub>Cl<sub>2</sub>. Boc-Phe-OH (0.796g, 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 17½ hours, followed by acetylation for 1 hour.

Deprotection of the phenylalanyl peptide was achieved using the usual 40% TFA/CH<sub>2</sub>Cl<sub>2</sub>. Boc-Pro-OH (0.646g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBt (0.92, 6mmol) for 2 hours followed by acetylation for 1 hour.

After deprotection Boc-His(Dnp)-OH (1.44g, 3 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 14½ hours followed by acetylation for 1 hour.

The resin was then washed with DMF (3X), CH<sub>2</sub>Cl<sub>2</sub> (3X) i PrOH (2X), CH<sub>2</sub>Cl<sub>2</sub> (3X) and finally MeOH (3X) and dried to give 3.5353g of product.

1.2g of this material was treated with HF at 0° for 1½ hours in the presence of anisol (1.5ml) then dried overnight over potassium hydroxide. The resin was then washed with DMF/ water (1:1), acetic acid and finally acetic acid/water (1:1) to remove the peptide. These washes were combined and evaporated in vacuo.

The residue was dissolved in DMF (15ml) and water (6 ml) thioethanol (5ml) added and the pH of the solution brought to

- 22 -

8.0 with sodium carbonate. The reaction was stirred overnight the solvent evaporated and the residue applied to a Sephadex G 25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 27-46 were combined and the solvent evaporated in vacuo and dried. Then 90% of the residue was taken (for the rest see Example III) and dissolved in anhydrous ammonia (100 ml) and small portions of sodium wire added until a permanent blue colour was achieved for 15 seconds. The ammonia was allowed to evaporate and the residue dried.

The residue was applied to a Sephadex SPC25 column (77 x 1.6 cms) eluted with 30 % acetic acid at 40 mls/hr, with a sodium chloride gradient from 0.01M to 1M over two days collecting 6.6 ml fractions.

The product was contained in fractions 100-104. These were pooled, evaporated and the residue dissolved in glacial acetic acid and filtered to remove the sodium chloride. The solution was evaporated and desalted on a Sephadex G25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr, collecting 6ml fractions. Fractions 32-6 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 13.4mg

<u>Product</u>	$C_{52}H_{74}O_9N_{12}$	MW.	1011,25
<u>T.l.c.</u> (silica)	Rf 0.15	EtOAc/Pyr/AcOH/H <sub>2</sub> O	40:20:6:11
	Rf 0.40	nBuOH/Pyr/AcOH/H <sub>2</sub> O	30:20:6:24
<u>T.l.e.</u>	pH 2.1	1000V	30 min mobility 8.3 cm.
	pH 6.5	1000V	30 min mobility 7.5 cm.
<u>AAA</u>	.6N HCl + phenol	110°	40 hours, peptide content 72%
His:1.97; Pro:1.01; Val:1.02; Tyr:0.98; Phe: 1.01.			

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Example IIH-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH (H-79)

Fractions 80-84 of the SPC 25 Sephadex column from the previous synthesis were combined, evaporated and the residue dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was evaporated and the product desalted on a Sephadex G25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 32 - 9 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 23.6 mg

<u>Product</u>	$C_{46}H_{67}O_8N_9$	MW	874,10	
<u>Tlc</u> (silica)	Rf 0.29	EtOAc/Pyr/AcOH/H <sub>2</sub> O	40:20:6:11	
	Rf 0.46	nBuOH/Pyr/AcOH/H <sub>2</sub> O	30:20:6:24	
<u>Tle</u>	pH 2.1	1000V	30 min	mobility 7.5 cms
	pH 6.5	1000V	30 min	mobility 8.3 cms
<u>AAA</u>	6N HCl + phenol,	110°, 40 hours,	peptide content	85%
His: 0.97; Pro: 1.08; Val: 0.99; Tyr: 0.97; Phe:1.00.				

The above example illustrates how Y in formulae (V), (VA) and (VB) may be absent.



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Example IIIH-His-Pro-Phe-His-Leu-reduced (SO<sub>2</sub>Ph)-Leu-Val-Tyr-OH (H-78)

In the synthesis of compound H76 10% of the residue from the Sephadex G25 column after the HF and thioethanol treatments of the resin was kept.

This material was applied to a Sephadex SPC25 column (77 x 1.6 cm) eluted with 30% acetic acid at 20 mls/hr with a sodium chloride gradient from 0.01M to 1M over 2 days collecting 6.6 ml fractions.

The product was contained in fractions 74-7. These were pooled, evaporated, dissolved in glacial acetic acid and filtered to remove sodium chloride. The solution was then evaporated and desalted on a Sephadex G25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 31-4 were pooled evaporated, the residue transferred to a vial and lyophilised.

Yield 0.6 mg

<u>Product</u>	C <sub>58</sub> H <sub>78</sub> O <sub>11</sub> N <sub>12</sub> S	MW:	1151.40
<u>Tlc(silica)</u>	Rf 0.31	EtOAc/Pyr/AcOH/H <sub>2</sub> O	40:20:6:11
<u>Tle</u>	pH 2.1	1000v 30 min	mobility 5.4 cms.
<u>AAA</u>	6N HCl + phenol, 40 hrs, 110°, peptide content 64%		
His: 1.93; Pro: 1.08; Val: 1.05; Tyr: 0.96; Phe: 0.97.			

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Example IVH-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH,

(H-77)

The Boc-Tyr [Bzl(2,6,Cl<sub>2</sub>)] -O-Resin (3g, 0.6mmol) was deprotected and Boc-Val-OH (0.65g, 3.0 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 22 hours\* then acetylated with acetylimidazole (0.66g, 6mmol) for 1 hour.

After deprotection, Boc-Leu-reduced (SO<sub>2</sub>Ph)-Leu-OH, IV, (0.42g 0.9 mmol) was coupled using DCCI (0.28g, 1.35 mmol) and HOBt (0.275g, 1.8 mmol) for 20 hours, then acetylated for 1 hour.

After deprotection Boc-His(Dnp)-OH (1.44g, 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 22 hours\*, then acetylated for 1 hour.

After deprotection, this time with 50% TFA/CH<sub>2</sub>Cl<sub>2</sub> Boc-Phe-OH (0.796g, 3 mmol) was coupled with DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 19 hours\* then acetylated for 1 hour.

After deprotection Boc-Pro-OH (0.646g, 3 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6 mmol) for 3 hours then acetylated for 1 hour.

The peptide was again deprotected and coupled with Boc-D-His(Boc)-OH (0.91g, 2.56 mmol using DCCI (0.58g, 2.82 mmol) and HOBt (0.78g, 5.1 mmol) for 2 hours, then acetylated for 1 hour.

The resin was washed with DMF (3X) CH<sub>2</sub>Cl<sub>2</sub> (3X) iPrOH (2X) CH<sub>2</sub>Cl<sub>2</sub> (3X) and finally MeOH (3X) and dried to give 3.6563 g of product.

1.2g of this material was treated with HF at 0° for 1½ hours in the presence of anisole (1.5 ml) then dried overnight over potassium hydroxide. The resin was then washed with DMF,

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DMF/H<sub>2</sub>O (1:1), acetic acid and finally acetic acid/water (1:1) to remove the peptide. These washes were combined and evaporated in vacuo.

The residue was dissolved in DMF (15ml) and water (6 ml), thioethanol (5ml) added and the pH of the solution brought to 8.0 with sodium carbonate solution. The reaction was stirred overnight, the solvent evaporated and the residue applied to a Sephadex G 25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6ml fractions. Fractions 27-46 were combined and the solvent evaporated in vacuo and dried.

90% of the residue was dissolved in anhydrous ammonia (100 ml) and small portions of sodium wire added until a permanent blue colour was achieved for 15 seconds. The ammonia was allowed to evaporate and the residue dried.

The residue was applied to a Sephadex SPC 25 column (77 x 1.6 cms) eluted with 30% acetic acid at 20 mls/hr with a sodium chloride gradient 0.01 M to 1M over 2 days collecting 6.6 ml fractions.

The product was contained in fractions 88-92. These were pooled, evaporated and the residue dissolved in glacial acetic acid and filtered to remove the sodium chloride. The solution was evaporated and desalted on a Sephadex G 25 column (72 x 2.5 cms) eluted with 50% acetic acid at 18 mls/hr collecting 6ml fractions. Fractions 32 - 41 were pooled, evaporated, transferred to a vial and lyophilised.

Yield 46.8 mg

Difference in yields between "L-His" and "D-His" compounds was accounted for by a lower incorporation of the isostere in the "L" case and less removal of the histidine with Na/NH<sub>3</sub>.

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Product  $C_{52}H_{74}O_9N_{12}$  MW 1011.25  
Tlc (silica Rf 0.18 EtOAc/Pyr/AcOH/H<sub>2</sub>O 40:20:6:11  
Tle pH 2.1 1000V 30 min mobility 7.7 cm  
pH 6.5 1000V 30 min mobility 7.9 cm  
AAA 6N-HCl + phenol, 110°, 40 hours, peptide content 93%  
His: 1.98; Pro:1.00; Val:1.08; Tyr: 0.97; Phe 0.97

EXAMPLE V

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH (H 113)

The method is generally that of Example I above but illustrates formula (VB).

Preparation of Boc-His (DNP)-O-Resin (AH/30/83)

Boc-His(DNP)-OH. (4.74g, 11.25 mmol) was dissolved in ethanol ( 60ml) and a solution of cesium bicarbonate (2.18g, 11.25mmol) in water (15 ml) added. The solvent was evaporated in vacuo and the residue treated four times with toluene and evaporated to remove water before finally drying overnight over phosphorus pentoxide. The residue was dissolved in DMF (175 ml), chloromethylated resin (30g, 22.5 m-equiv.) added and the reaction stirred at 37° for five days.

The resin was filtered off and washed thoroughly with DMF, DMF/water (9:1) and then DMF again. It was resuspended in DMF (175 ml) and treated with acetic anhydride (7.08 ml, 75 mmol) and triethylamine (10.5ml, 75 mmol) overnight.

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The resin ester was filtered, washed thoroughly with DMF, DMF/water (9:1) and methanol and dried. It was then "de-fined" by shaking it in dichloromethane and removing the particles in the supernatant. Finally, the resin was dried. Yield: 30.45g. A trial coupling with Boc-Ala-OH, followed by amino-acid analysis (after hydrolysis with 12N-HCl/propionic acid 1:1, 130°, 2hrs) gave an incorporation of 0.22mmol/.

#### Coupling to Resin Ester

Boc-His(DNP)-O-Resin (2.5g 0.55 mmol) was deprotected with 50% TFA/CH<sub>2</sub>Cl<sub>2</sub> and Boc-Ile-OH (0.748g 3mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6.0mmol) for 2 hours, then acetylated with acetyl imidazole (0.55g, 5mmol) overnight\*.

After deprotection with 40% TFA/CH<sub>2</sub>Cl<sub>2</sub>, Boc-Leu-reduced (3,4-Cl<sub>2</sub>-Z)-Val-OH, 4 (0.343g, 0.66 mmol) was coupled using DCCI (0.15g, 0.73mmol) and HOBt (0.202g, 1.32 mmol) for 16 hours, then acetylated for 1 hour.

After deprotection, Boc-His(DNP)-OH (1.26g, 3.0 mmol) was coupled using DCCI (0.68g, 3.3 mmol) and HOBt (0.92g, 6mmol) for 2 hours, then acetylated for 1 hour.

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After deprotection, again with 50% TFA/ $\text{CH}_2\text{Cl}_2$ , Boc-Phe-OH (0.796g, 3mmol) was coupled with DCCI (0.68g, 3.3mmol) and HOBT (0.92g, 6mmol) for 3 hours\* then acetylated overnight\*.

After deprotection, Boc-Pro-OH (0.646g, 3mmol) was coupled using DCCI (0.68g, 3.3mmol) and HOBT (0.92g, 6mmol) for 2 hours then acetylated for 1 hour.

The peptide was again deprotected and coupled with Boc-His (DNP)-OH (1.26g, 3mmol) using DCCI (0.68g, 3.3mmol) and HOBT (0.92g, 6mmol) for 2 hours, then acetylated overnight\*.

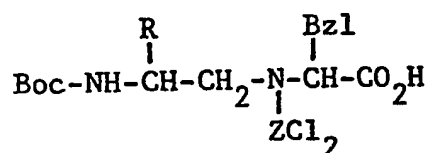
The peptide resin ester was washed with DMF (3X),  $\text{CH}_2\text{Cl}_2$  (3X) iPrOH (2X),  $\text{CH}_2\text{Cl}_2$  (3X) and finally MeOH (3X) and dried. It was then treated with HF at  $0^\circ$  for  $1\frac{1}{2}$  hours in the presence of anisole (4ml) and dried overnight over potassium hydroxide. The resin was washed with DMF, acetic acid and acetic acid/water (1:1) to remove the peptide. The washes were combined and evaporated in vacuo.

The residue was dissolved in DMF (60 ml) and water (24ml), thioethanol (10ml) was added and the pH of the solution brought to 8.0 with sodium carbonate solution. The reaction mixture was stirred overnight, the solvent evaporated and the residue applied to a Sephadex G25 column (77 x 2.5cms). It was eluted with 50% acetic acid at 18 mls/hr collecting 6 ml fractions. Fractions 34-53 were combined and the solvent evaporated in vacuo and dried.

Product  $\text{C}_{49}\text{H}_{72}\text{O}_3\text{N}_{14}$  MW 985,21  
 Tlc (silica) Rf: 0.63 in EtOAc-Py-AcOH- $\text{H}_2\text{O}$  (15:20:6:11)  
 AA analysis in accordance with calculated composition.

EXAMPLES VI - IX

These Examples illustrate formula (VA). The methods disclosed above are applied to condensing Boc-Phe-H or Boc-Leu-H with H-Phe-OBzl, reducing the imine link, deprotecting at the carboxyl terminus and protecting the nitrogen of the reduced peptide link to give:



12 R = Bzl

12a R = iBu                      ZCl<sub>2</sub> = 3, 4-dichloro benzyloxy carbonyl

This Phe-reduced-Phe or Leu-reduced-Phe analogue is then used as follows:

- VI Use of 12 (Phe-reduced-Phe) in an analogue otherwise as H-77 (see Example IV)
- VII Use of 12a (Leu-reduced-Phe) in an analogue otherwise as H-77 (see Example IV)
- VIII Use of 12 in an analogue as H-76 (Example I), viz:

H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH (H 110)

6 7 8 9 10 11 12 13

- IX Use of 12a in an analogue as H-76 (Example I), viz:

H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH (H 115)

6 7 8 9 10 11 12 13

EXAMPLE X

This Example illustrates formula (VB), the method of Example V being used but with the Tyr resin of Examples I to IV, to give:

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH (H 116)  
           6   7   8   9   10              11  12  13

EXAMPLE XI

Use of the methods disclosed herein to give:

H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH (H 108)  
           5   6   7   8   9   10              11  12  13  14

this being an analogue illustrating the non-criticality of the terminal portions of the chain allowing X and W in formulae (V), (VA) and (VB) to represent further residues. It is a further example of formula (VA).

EXAMPLE XII

Use of the methods disclosed herein to give:

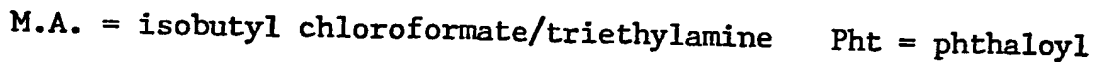
H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH (H 117)  
           6   7   8   9   10              11  12  13

a compound of value in its relation to the Leu-Val structure at 11, 12 in human renin substrate.



### EXAMPLE XIII

### Scheme 4



The resulting N-terminal phthaloyl protected, -OH protected hydroxy isostere of Leu-Leu can be coupled direct for example to valyl tyrosine, followed by removal of the phthaloyl group, coupling direct to a suitable tri or tetrapeptide, and deprotection at the -OH group by mild acid hydrolysis, to give for example analogues corresponding to H-76 (Example 1), H-79 (Example 2), H-77 (Example 4). Alternatively the phthaloyl group may be removed by treatment with hydrazine and a new protective group, e.g. benzyloxycarbonyl or t-butyloxycarbonyl attached prior to coupling.

The methods used after the preparation of the protected hydroxy isostere are those of the peptide synthesis art, well known in themselves and exemplified in detail herein. The compounds specifically prepared are:

- a) H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH
- b) H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH
- c) H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH

#### EXAMPLE XIV

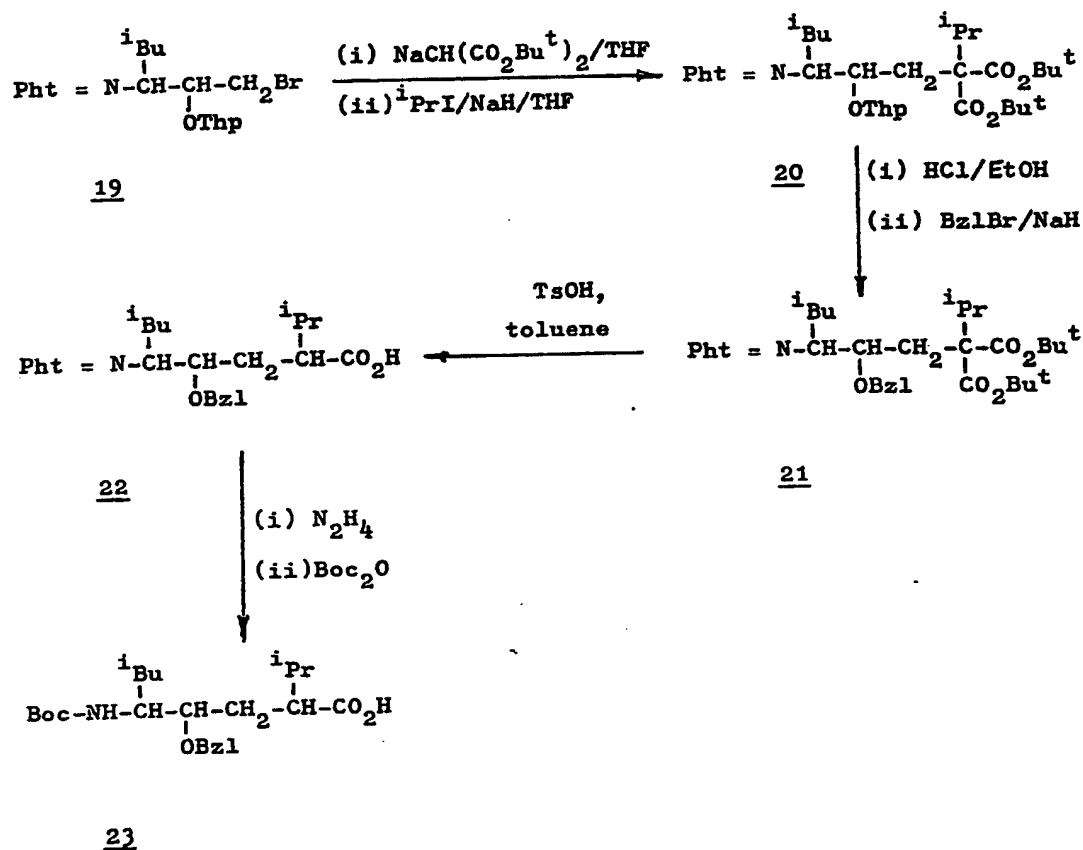
The alternative and preferred reaction scheme below, also suitable for other dipeptide hydroxy isosteres was used to synthesise an N-terminal and hydroxy-group protected Leu-Val hydroxy isostere 23.

-34-

Scheme 5

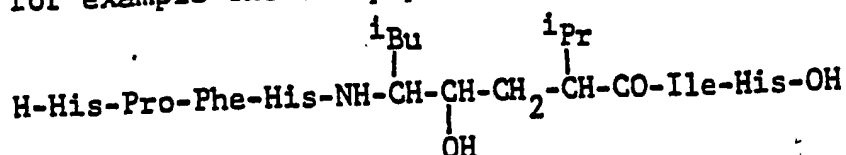
Pht = Phthaloyl,

Thp = Tetrahydropyranyl



In the above scheme the protected bromohydrin 19 is obtained in the same way as the corresponding intermediate 17 in Scheme 4, and is subjected to malonic ester synthesis and alkylation with isopropyl iodide to give the malonic ester derivative 20. Protection on the hydroxyl function is changed from Thp to Bzl to yield 21 and the latter is subjected to protonolysis and decarboxylation. In the resulting isostere acid 22 amino protection is changed from Pht to Boc yielding the protected isostere 23 which is suitable for incorporation into an octapeptide analogue by the usual methods of solid phase peptide synthesis to

give for example the octapeptide analogue:

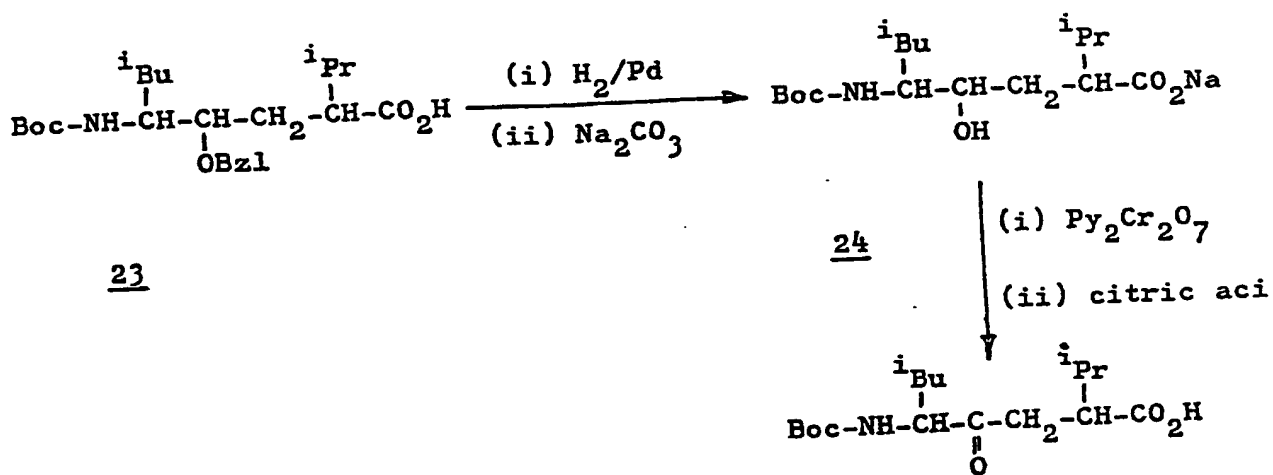


This is a Leu-hydroxy-Val isostere.

#### EXAMPLE XV

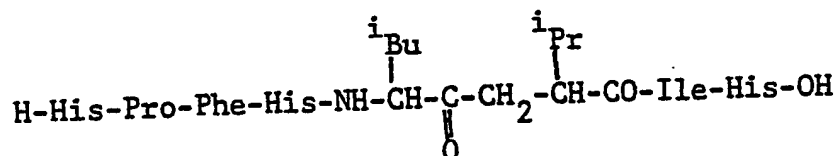
Keto isosteres may be prepared for example by the method of published U.K. Specification No. 1 587 809 (U.S. 4 242 256) of R. Sharpe and one of the present inventors M. Szelke, to which reference may be made. Alternatively they may be prepared from hydroxy isosteres prepared as disclosed herein and in particular, in the present Example, from the final product 23 of scheme 5 as in the scheme below:-

#### Scheme 6



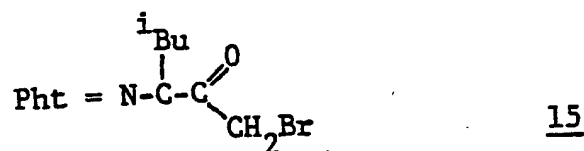
In the above scheme the benzyl protection is selectively removed from the hydroxyl function of 23 and the free acid is converted into its sodium salt 24. The latter is subjected

to oxidation by pyridinium dichromate and acidification to give the partially protected keto isostere acid which is ready for incorporation into an octapeptide analogue by the usual methods of solid phase synthesis, for example the analogue:

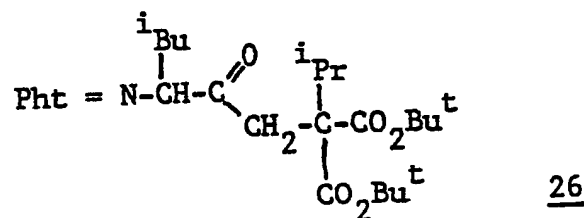


This is a Leu-keto-Val isostere.

Alternatively the keto isostere may be prepared directly by a modified version of scheme 5 wherein the bromohydrin 19 is replaced by the bromoketone 15 (scheme 4), which is:

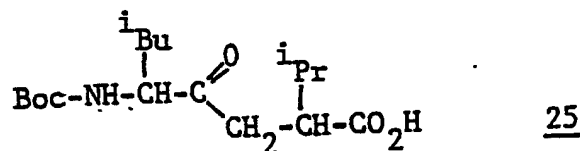


giving the compound



which is successively treated with TsOH, toluene and i)  $\text{N}_2\text{H}_4$ ,

ii)  $\text{Boc}_2\text{O}$  to give:



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ACTIVITY IN VITRO

Preliminary activity test results in the human renin-renin substrate reaction in vitro are given in the table below, with comparative figures for the tetrapeptide analogue (III). The test is based on the methods described by J.A. Millar et al. in Clinica Chimica Acta (1980) 101 5-15 and K. Poulsen and J. Jorgensen in J. Clin. Endocrinol. Metab. (1974) 39 816.

It is based on the measurement, by radioimmunoassay, of Angiotensin-I released from human renin substrate by human renin in human plasma. The inhibitor is dissolved in 0.01 N HCl (10  $\mu$ l) and added to human plasma (75  $\mu$ l) containing EDTA, and angiotensin-I antibody (15  $\mu$ l) in 3M-Tris/HCl buffer (pH 6.9).

After incubation at 37°C for 0-120 mins., the enzymic reaction is quenched by the addition of ice-cold 0.25M Tris/HCl buffer (pH 7.4) containing 0.01% of bovine serum albumin. 125I-labelled angiotensin-I is added, followed by equilibration at 4°C for 48 hours. Free and bound ligand are separated by the addition of dextran-coated charcoal, and the amount of bound radio-ligand determined in a gamma counter.

The results for the renin inhibitory activities of the present compounds thus tested, expressed as the IC<sub>50</sub> (the molar concentration required to cause 50% inhibition), are as follows:

<u>Analogue</u>	<u>IC<sub>50</sub></u>
Example I (H-76)	1.0 $\mu$ M
Example II (H-79)	17.0 $\mu$ M
Example III (H-78)	1.5 $\mu$ M
Example IV (H-77)	1.0 $\mu$ M
Example V (H-113)	0.26 $\mu$ M **
Example VI	*
Example VII	*
Example VIII (H-110)	1.3 $\mu$ M
Example IX (H-115)	2.5 $\mu$ M
Example X (H-116)	0.20 $\mu$ M
Example XI (H-108)	0.05 $\mu$ M
Example XII (H-117)	*
Example XIII a)	*
b)	*
c)	*
Example XIV	*
Example XV	*

\* Preliminary indication of comparable activities.

\*\* The corresponding non-isosteric peptide has for example been tested and shows a potency over three orders of magnitude less (IC<sub>50</sub> 400  $\mu$ M)

(Comparative) III 822  $\mu$ M

These are most notable results, showing a potency, in the reduction of renin activity remaining in the plasma in the presence of the analogue, several orders of magnitude greater than the previously proposed tetrapeptide analogue.

ACTIVITY IN VIVO

The activity tests below are in animals but indicate corresponding activity in man.

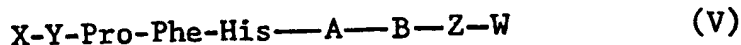
In in vitro studies, in plasma from both normal and sodium-depleted dogs, the compound H-77 (Example IV) inhibited renin. In in vivo studies H-77 was infused into normal conscious sodium-depleted dogs at rates of 0.01, 0.1, 1 and 10 mg/kg/hr. A maximum fall in blood pressure, plasma renin (PR) angiotensin-I (A-I) and angiotensin-II (A-II) levels was obtained within 10 minutes at doses of 1 and 10 mg/kg/hr. When the infusion was stopped, blood pressure returned to baseline levels 30 minutes after the 1 mg/kg/hr. dose, but more slowly after the 10 mg/kg/hr. dose.

In the claims below it will be understood that compounds may be in the form shown or in protected or salt form at  $\text{NH}_2$ ,  $\text{COOH}$ ,  $\text{OH}$  or other active groups and in particular as their physiologically acceptable acid addition salts at basic centres. Further as already noted herein general reference to amino acids and amino acyl residues and side chains is to be taken as reference to such whether naturally occurring in proteins or not and to both D- and L- forms, and amino is to be taken as including imino except where an aromatic acid, residue or side chain is specified.



CLAIMS:

1. A polypeptide analogue of the formula:



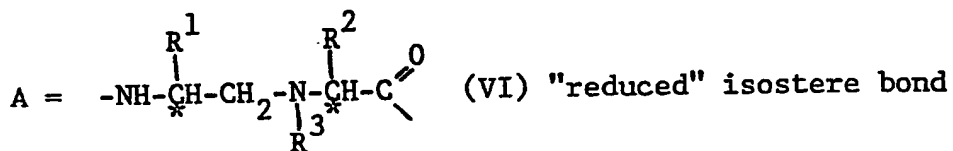
6 7 8 9 10,11 12 13

where:-

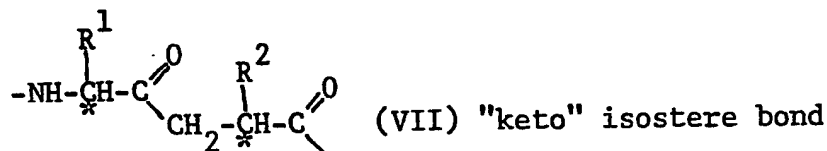
Pro, Phe and His may be in substituted form;

X = H; or an acyl or other N-protecting group e.g. acetyl, pivaloyl, *t*-butyloxycarbonyl (Boc), benzoyl or lower alkyl (primarily C<sub>1</sub>-C<sub>5</sub>); or an L- or D-amino-acyl residue, which may itself be N-protected similarly;

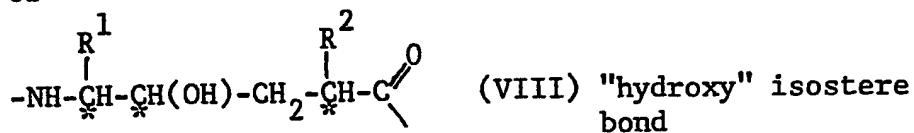
Y = D- or L-His or other D- or L- basic or aromatic amino-acyl residue, or is absent;



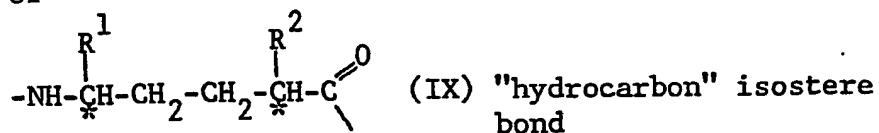
or



or



or



where the configuration at asymmetric centres \* is either

R or S, where in VIII the hydroxy group may be present as such or protected in ether  $-OR^4$  or ester  $-O-C(=O)R^4$  form

where  $R^4$  is as given under W below and where

$R^1$  and  $R^2$ , the same or different =  $^i\text{Pro}$  (isopropyl),

$^i\text{Bu}$  (isobutyl), Bzl (benzyl) or other lipophilic or aromatic amino-acid side chain

$R^3$  =  $-H$ ; lower alkyl ( $C_1-C_5$ ); or  $-\text{SO}_2\text{Ph}$ ,  $-\text{SO}_2\text{C}_6\text{H}_4\text{CH}_3(p)$ ,

Boc, formyl or other N-protecting group;

B = D- or L- Val or Ile or other D- or L- lipophilic amino-acyl residue;

Z = D- or L- Tyr, Phe, His or other L- or D-aromatic amino-acyl residue;

and

W =  $-OH$  as such or in protected ester form as  $-OR^4$  where

$R^4$  = lower alkyl primarily  $C_1-C_5$  and particularly

$^t\text{Bu}$ , or cycloalkyl primarily  $C_3-C_7$ , or Bzl, or other ester forming group; or  $-\text{NH}_2$  as such or in protected

amide form as  $-\text{NHR}^5$  or  $-\text{N}(\text{R}^5)_2$  (where  $\text{R}^5$  = an

N-protecting or other substituent group e.g. lower alkyl as for  $R^4$  and  $(\text{R}^5)_2$  = two such or e.g. cycloalkyl, primarily  $C_3-C_7$ ) or as  $-\text{NH}-(\text{CH}_2)_n-\text{Q}$  or

$-\text{NR}^5-(\text{CH}_2)_n-\text{Q}$  (where  $n = 2$  to  $6$  and  $\text{Q} = \text{NH}_2$  or

$-\text{NH}-\text{C}(=\text{NH})\text{NH}_2$  and wherein any of the hydrogens attached to

nitrogen may be substituted by  $\text{R}^5$  or  $(\text{R}^5)_2$ ; an L- or D-

serine or lysine, arginine or other basic amino-acyl residue as such or in amide form, substituted amide form or ester form e.g. containing a group or groups as given for  $R^4$  and  $R^5$  above as the case may be; or an amino alcohol residue derived therefrom as such or protected in ester or ether form e.g. containing a group as given for  $R^4$  above

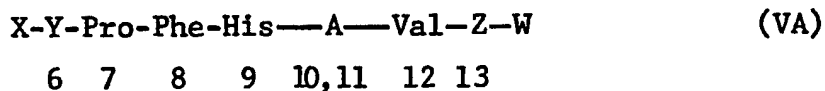
or

L- or D-

Z + W = an alcohol derived from/Tyr, Phe, His or other L- or D- aromatic amino-acyl residue as such or protected in ester or ether form as above;

such polypeptide being in the above form or modified by isosteric replacement of one or more remaining peptide bonds by reduced,  $-\text{CH}_2-\text{NH}-$ , keto,  $-\text{C}(=\text{O})-\text{CH}_2-$ , hydroxy,  $-\text{CH}(\text{OH})-\text{CH}_2-$ , or hydrocarbon,  $-\text{CH}_2-\text{CH}_2-$  isosteric links and further being in free form or in protected form at one or more remaining peptide, carboxyl, amino, hydroxy or other reactive groups.

2. A polypeptide analogue, according to claim 1, of the formula:



where

X, Y, Pro, Phe and His are as in claim 1

A is as in claim 1 except that

$R^1$  and  $R^2$ , the same or different =  $^i\text{Bu}$  (isobutyl)  
or Bzl (benzyl) or other lipophilic or aromatic  
amino-acid side chain

$R^3 = -H$ ; or  $-SO_2Ph$ ,  $-SO_2C_6H_4CH_3(p)$ , Boc, formyl

or other N-protecting group

Z = Tyr, Phe or other L- or D-aromatic amino-acyl residue;

W =  $-OH$  as such or in protected ester form as  $-OR^4$  where

$R^4 =$  lower alkyl (primarily  $C_1-C_5$  and particularly

$tBu$ ), or Bzl, or other ester forming group ; or

$-NH_2$  as such or in protected amide form as  $-NHR^5$  or

$-N(R^5)_2$  ( $R^5 =$  an N-protecting group e.g. lower alkyl

as for  $R^4$ ;  $(R^5)_2 =$  two such or e.g. cyclo-alkyl,

primarily  $C_3-C_7$ ) or

an L- or D- amino-acyl residue e.g. a serine or basic amino-acyl residue as such or in amide form or in

protected amide or ester form e.g. containing a group

or groups as given for  $R^4$  and  $R^5$  above as the case

may be; or an amino acid alcohol residue derived

therefrom as such or protected in ester or ether form

e.g. containing a group as given for  $R^4$  above

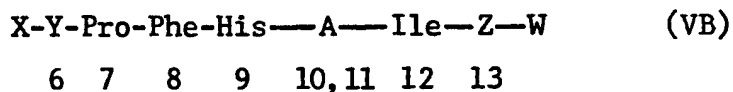
or

Z + W = an alcohol derived from Tyr or Phe or other L-

or D- aromatic amino acyl residue as such or protected

in ester or ether form as above.

3. A polypeptide analogue, according to claim 1, of the formula:



where

X, Y, Pro, Phe and His are as in claim 1

A is as in claim 1 except that

$R^1 = i\text{Bu}$  (isobutyl) or Bzl (benzyl) or other  
lipophilic or aromatic amino-acid side chain

$R^2 = i\text{Pr}$  (isopropyl), and

$R^3 = -\text{H}$ ; or  $-\text{SO}_2\text{Ph}$ ,  $-\text{SO}_2\text{C}_6\text{H}_4\text{CH}_3(\text{p})$ , Boc, formyl

or other N-protecting group

Z is as in claim 1

W is as in claim 2

or Z + W = an alcohol derived from the aromatic residues  
specified for Z in claim 1, as such or protected in ester  
or ether form as specified therein.

4. A polypeptide according to any one of claims 1 to 3,  
modified by isosteric replacement, as set out therein, at one  
or both of the Pro-Phe or Phe-His links.

5. A polypeptide according to any one of claims 1 to 4  
wherein the isosteric replacement at at least the 10,11 position  
is of the "reduced" kind.

6. A polypeptide according to any one of claims 1 to 4  
wherein the isosteric replacement at at least the 10,11 position  
is of the "hydroxy" kind.

7. The compound:

H-His-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

8. The compound:

H-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

9. The compound:

H-His-Pro-Phe-His-Leu-reduced (SO<sub>2</sub>Ph)-Leu-Val-Tyr-OH.

10. The compound:

H-DHis-Pro-Phe-His-Leu-reduced-Leu-Val-Tyr-OH.

11. The compound:

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-His-OH.

12. The compound:

H-DHis-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH.

13. The compound:

H-DHis-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH.

14. The compound:

H-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-OH.

15. The compound:

H-His-Pro-Phe-His-Leu-reduced-Phe-Val-Tyr-OH.

16. The compound:

H-His-Pro-Phe-His-Leu-reduced-Val-Ile-Tyr-OH.

17. The compound:

H-Pro-His-Pro-Phe-His-Phe-reduced-Phe-Val-Tyr-Lys-OH.

18. The compound:

H-His-Pro-Phe-His-Leu-reduced-Val-Val-Tyr-OH.

19. The compound:

H-His-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

20. The compound:

H-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

21. The compound:

H-DHis-Pro-Phe-His-Leu-hydroxy-Leu-Val-Tyr-OH.

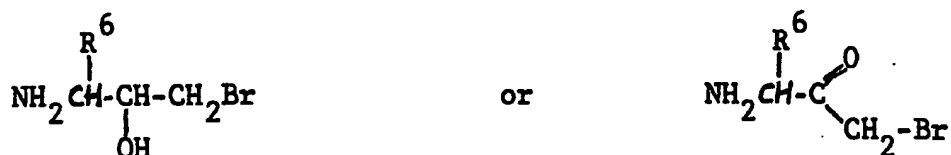
22. The compound:

H-His-Pro-Phe-His-Leu-hydroxy-Val-Ile-His-OH.

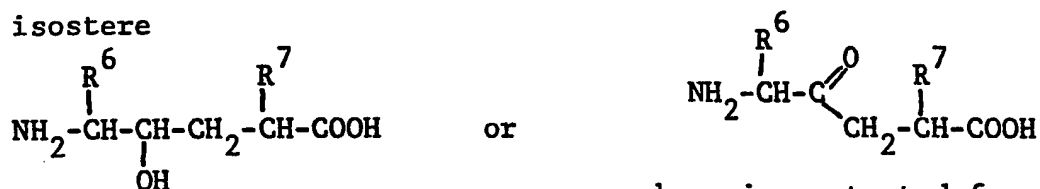
23. The compound:

H-His-Pro-Phe-His-Leu-keto-Val-Ile-His-OH.

24. A method of making a hydroxy or keto isostere of a dipeptide wherein a derivative of a halohydrin preferably a bromohydrin or haloketone preferably a bromoketone



wherein  $\text{R}^6$  is an amino acid side chain and the  $\text{NH}_2$  and OH groups are in protected form is alkylated to give the desired isostere



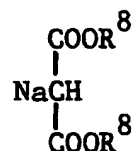
as such or in protected form,  $\text{R}^7$  being the same or a different amino acid side chain.

25. A method according to claim 24 wherein the alkylation procedure is by reaction with an alkali metal carboxylic acid derivative preferably a lithium derivative



where  $\text{R}^7$  is as in claim 24.

26. A method according to claim 25 wherein the alkylation procedure is by reaction with an alkali metal malonic ester derivative preferably a sodium derivative



where  $\text{R}^8$  is an esterifying group and a halide preferably an



-48-

iodide



where  $R^7$  is as in claim 24 to give intermediate

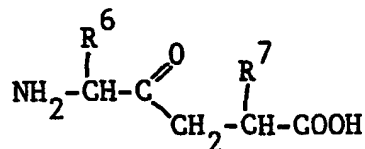


in protected form which intermediate is then decarboxylated and if desired deprotected to give the desired isostere



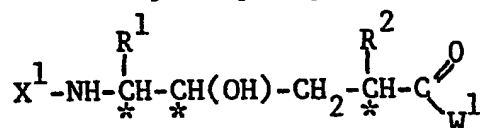
as such or in protected form.

27. A method of making a keto isostere of a dipeptide wherein a hydroxy isostere is made by the method of any of claims 24 to 26 and the  $-\text{CH}(\text{OH})-$  group is oxidised to give the desired isostere



as such or in protected form.

28. The method of any of claims 24 to 27 when applied to the production of a hydroxy dipeptide isostere of the formula



or the corresponding keto isostere, where the significance of \*,  $X^1$  and  $W^1$  is as in claim 1 except that  $X^1$  and  $W^1$  do not represent amino-acyl.

29. A hydroxy or keto isostere of a dipeptide when made by the method of any of claims 24 to 28, as such or in the form of a higher polypeptide analogue made using it.

30. In a diagnostic test for high-renin states, blood pressure falling most when renin is high, and as a surgical prognostic test for reno-vascular hypertension (renal artery stenosis), the administration of a polypeptide analogue according to any one of claims 1 to 23 followed by monitoring of blood pressure, and such polypeptide analogues when for such use.

31. In the long and short term treatment of heart failure and all forms of hypertension particularly those associated with high serum renin levels, the administration of a renin-inhibiting amount of a polypeptide analogue according to any one of claims 1 to 23, and such polypeptide analogues when for such use.

32. Administration according to claim 30 or 31, of 0.001 to 10 mg/kg body weight daily, preferably 0.01 to 1.0 mg, of the polypeptide analogue.

33. A polypeptide analogue according to any one of claims 1 to 23, when in the form of a composition with a pharmaceutically acceptable diluent or carrier.

34. The composition of claim 33, in unit dosage form containing the amounts of analogue set out in claim 32 or sub-multiple thereof.



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**PARTIAL EUROPEAN SEARCH REPORT**  
which under Rule 45 of the European Patent Convention  
shall be considered, for the purposes of subsequent  
proceedings, as the European search report

**0045665**

Application number

EP 81 30 3585.4

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D	<p>US - A - 4 198 398 (HUDSON et al.) * column 4, line 64 to column 7, line 45 *</p> <p>—</p> <p>DE - A1 - 2 714 141 (NATIONAL RESEARCH DEVELOPMENT CORP.) * claims; examples 1 to 6 *</p> <p>—</p> <p>DE - A1 - 2 811 267 (NATIONAL RESEARCH DEVELOPMENT CORP.) * claims; example *</p> <p>—</p>	<p>24,31, 33</p> <p>24,31, 33</p> <p>24,31, 33</p>	<p>C 07 C 103/52</p> <p>C 07 C 101/30</p> <p>C 07 C 101/34</p> <p>A 61 K 37/02</p> <p>//C 07 C 101/04</p> <p>C 07 C 101/24</p>
A	<p>Chemical Abstracts vol. 75, no. 23, December 1971</p> <p>Columbus, Ohio, USA</p> <p>K. SHIGEZANE et al. "Synthesis of anti-renin active peptides. II."</p> <p>page 350, abstract no. 141149r</p> <p>&amp; Yakugaku Zasshi, vol. 91, no. 9, 1971, pages 987 to 996</p> <p>—</p>	1-23	<p>C 07 C 101/30</p> <p>C 07 C 101/34</p> <p>C 07 C 103/52</p>
<p>—</p> <p>./..</p>			
<p><b>INCOMPLETE SEARCH</b></p> <p>The Search Division considers that the present European patent application does not comply with the provisions of the European Patent Convention to such an extent that it is not possible to carry out a meaningful search into the state of the art on the basis of some of the claims.</p> <p>Claims searched completely: 1-31, 33</p> <p>Claims searched incompletely: —</p> <p>Claims not searched: 32, 34</p> <p>Reason for the limitation of the search:</p> <p>Method for treatment of the animal or human body by therapy</p>			<p><b>CATEGORY OF CITED DOCUMENTS</b></p> <p>X: particularly relevant</p> <p>A: technological background</p> <p>O: non-written disclosure</p> <p>P: intermediate document</p> <p>T: theory or principle underlying the invention</p> <p>E: conflicting application</p> <p>D: document cited in the application</p> <p>L: citation for other reasons</p> <p>&amp;: member of the same patent family, corresponding document</p>
Place of search	Date of completion of the search	Examiner	
Berlin	05-10-1981	BREW	

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## PARTIAL EUROPEAN SEARCH REPORT

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DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int. Cl.)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
A	Chemical Abstracts vol. 85, no. 17 October 1976 Columbus, Ohio, USA M.J. PARRY et al. "Bio-isosteres of a peptide renin inhibitor" page 65, abstract no. 117050n & Chem. Biol. Pept., Proc. Am. Pept. Symp. 3rd, 1972, pages 541 to 544	1-31, 33	
	Chemical Abstracts vol. 80, no. 1, January 1974 Columbus, Ohio, USA I. PARIKH et al. "Substrate analog competitive inhibitors of human renin" page 108, abstract no. 1086w & Biochem. Biophys. Res. Commun., vol. 54, no. 4, 1973, pages 1356 to 1361	1-23	TECHNICAL FIELDS SEARCHED (Int. Cl.)
A	Chemical Abstracts vol. 87, no. 8, July 1977 Columbus, Ohio, USA K. POULSEN et al. "Competitive inhibitors of renin. A review." page 139, abstract no. 1561s & Prog. Biochem. Pharmacol., vol. 12, 1976, pages 135 to 141	1-23	